# LKB 4800A ULTROTOME® PG

PRIEUR
Please return to
DJ Prieur
David J. Prieur

## OPERATION MANUAL

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LKB-PRODUKTER AB
P. O. Box 12220, Stockholm 12, Sweden

Type LKB 4800

ULTROTOME

# PICTORIAL GUIDE

## Condensed Operation Manual

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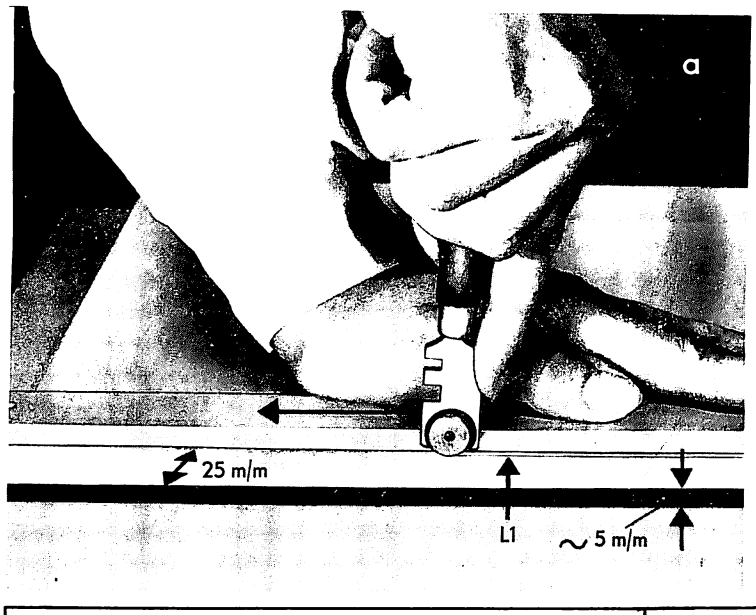
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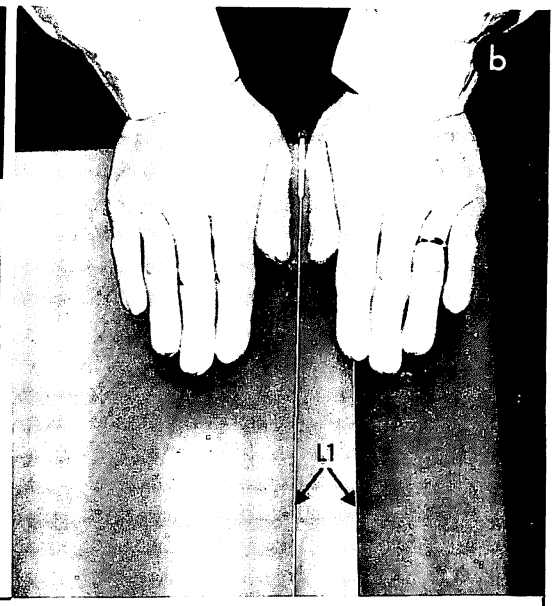
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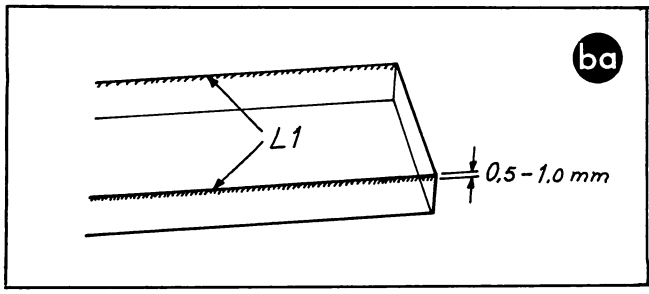
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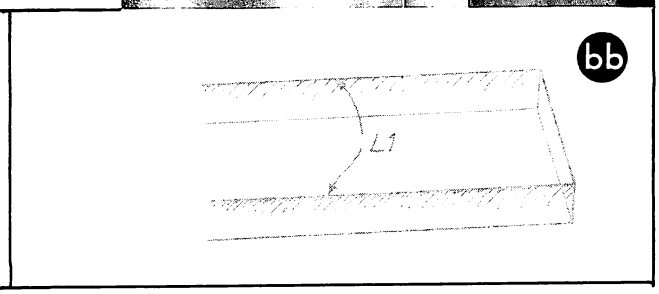
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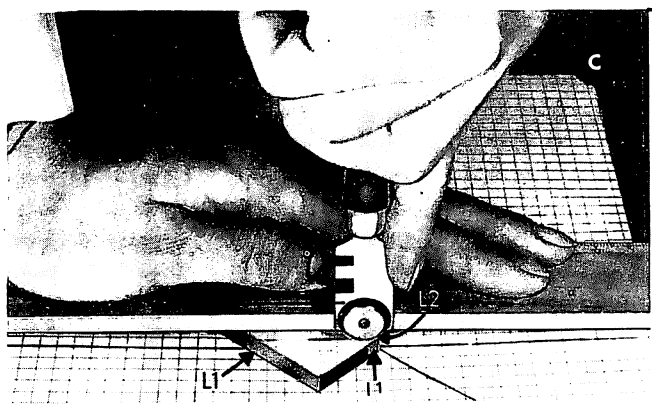
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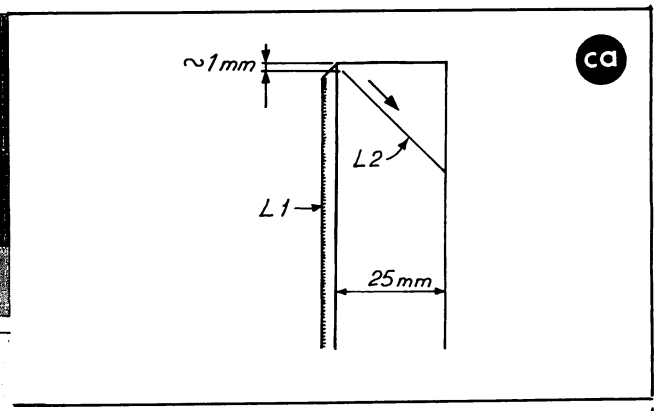
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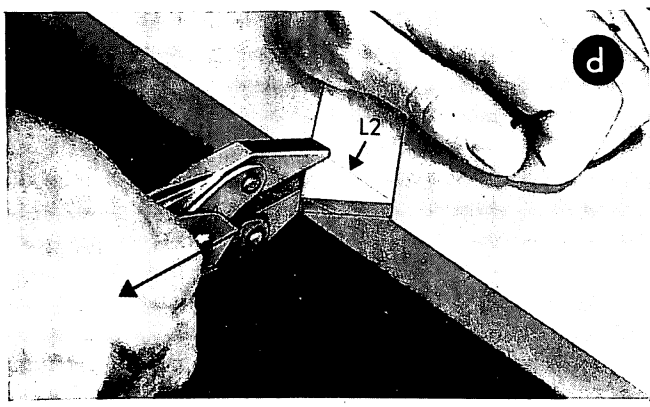
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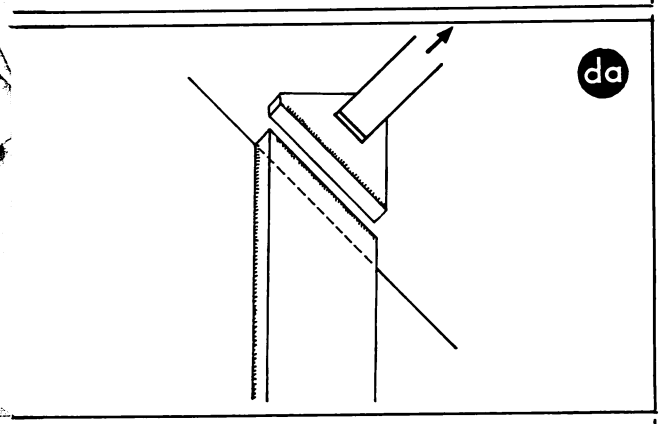
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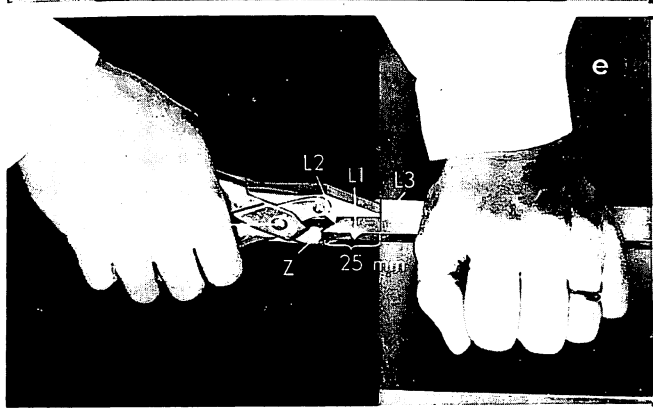
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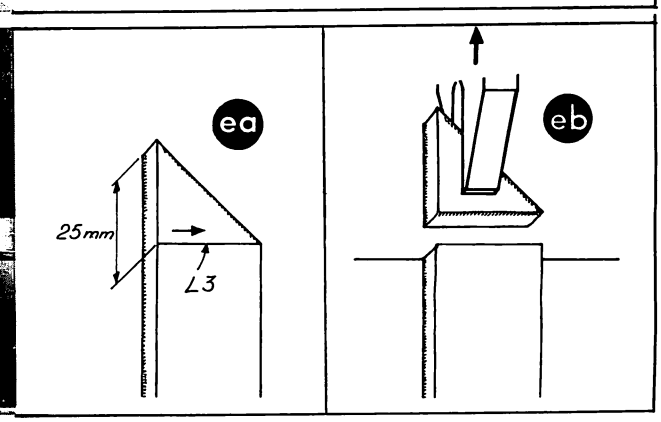
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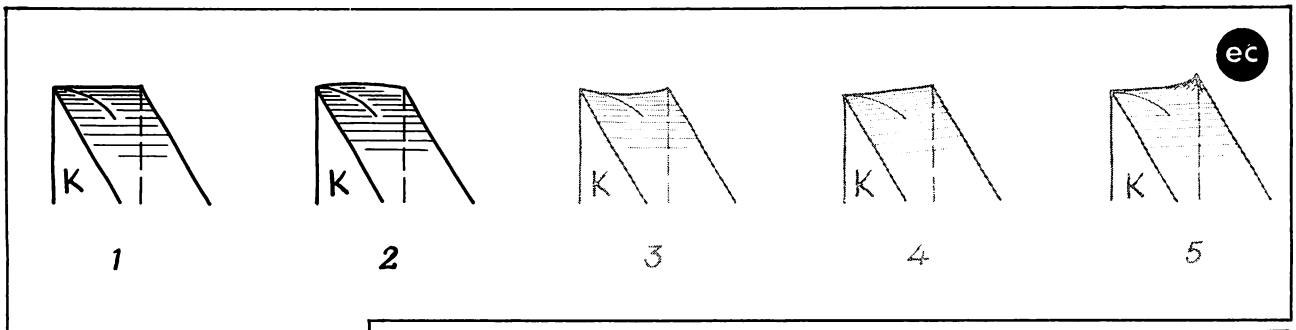
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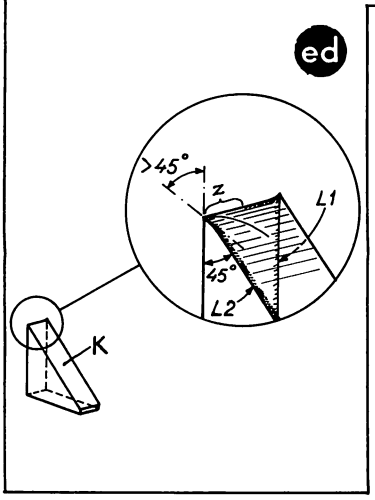
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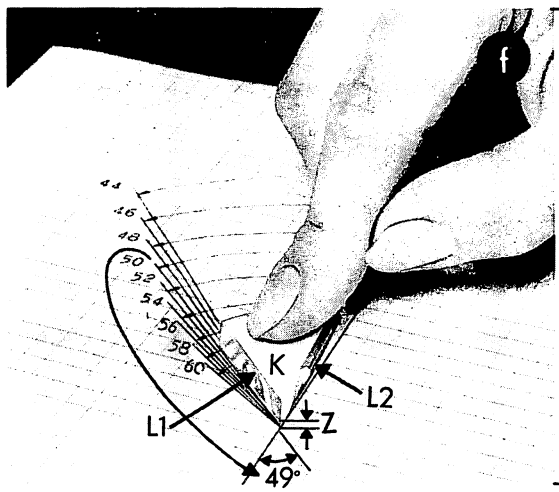
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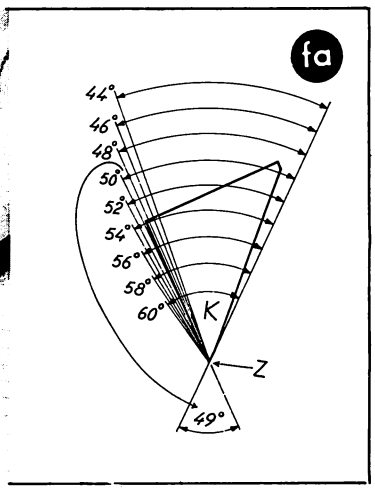
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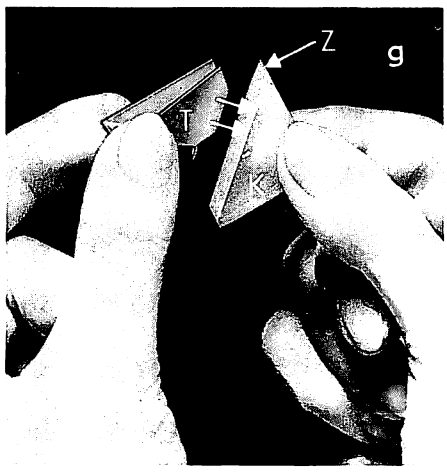
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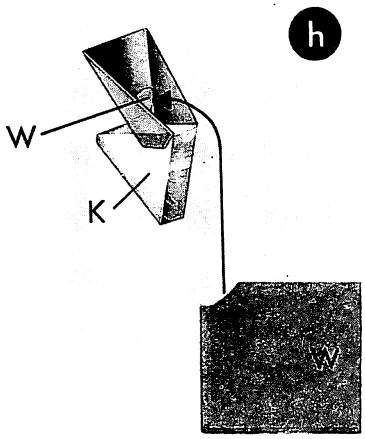
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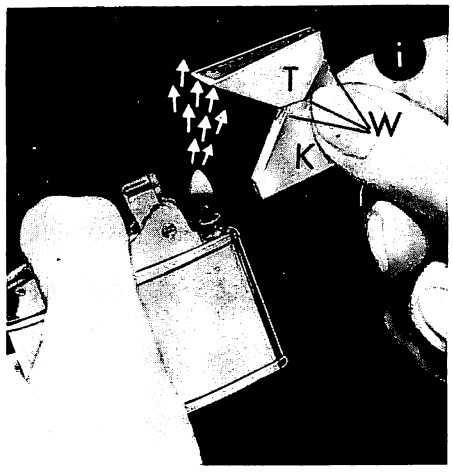
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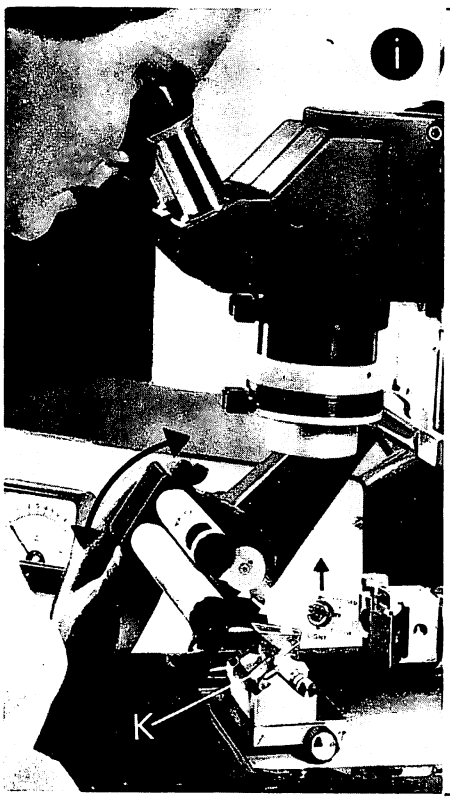
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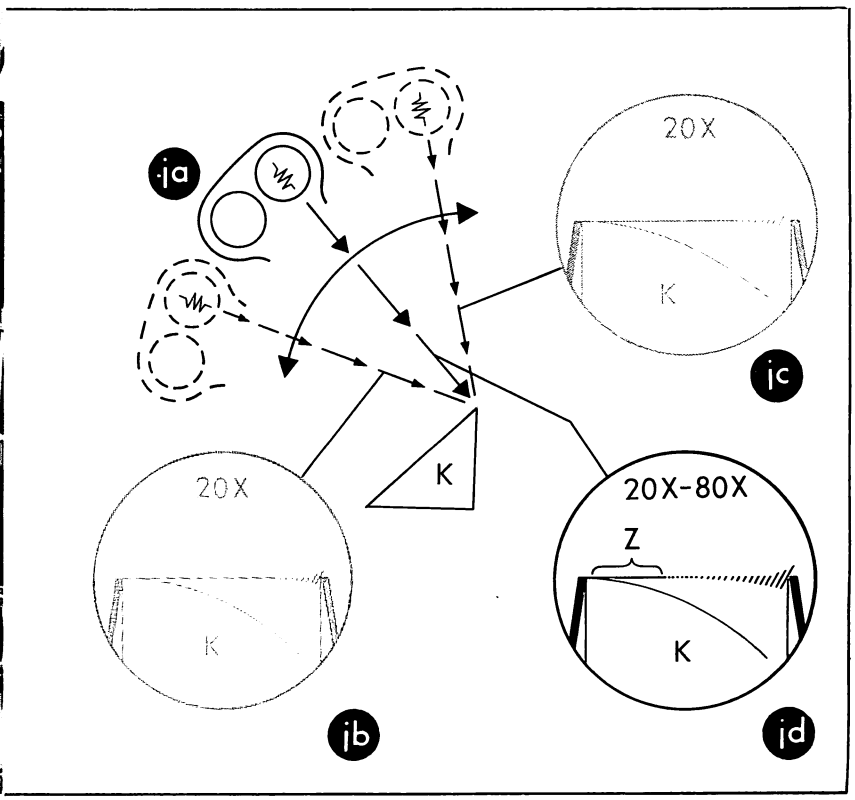
ia
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20X-80X
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id

# Specimen Trimming

*The following text refers to illustrations marked VII B 1: a–fb*

**a–c Equipment Needed:**

**a** Microscope on stand with focused light.
Hand supporting pad.
Specimen holder in specimen orientation head or trimming stand.

**b** Specimen block (M) containing specimen (Y).
**Note:** Before clamping the block in the holder the gleatine capsule in which it is cast must be removed.

**ba** 5 different sizes of specimen holders (marked B–G followed by a serial number) corresponding to capsule sizes 4–00.

**c** One quarter of a normal double-edged razor blade or half a single-edged "Shick" blade (R) mounted in holder (U).

**d–e Shaping and Orientating the Specimen Tip**
This may be performed either on front part of the Ultrotome or on a separate support, e.g. on wooden accessory box. Specimen holder may be either fastened in head or placed *on* or *in* trimming stand. Generally, the orientation head is especially useful for trimming oblique pyramids – the trimming stand can only be used for trimming straight pyramids. By "straight" is meant a pyramid or other shape which has its axis parallel to the specimen holder's axis.

The head is placed directly on Ultrotome front plane or on lid of wooden box.
Specimen holders with blocks which can be trimmed without the aid of an orientation head are placed *in* the trimming stand's deep recess with the shaft in the through hole when working on Ultrotome and *on* shallow recessed area but not the hole when trimming on the box (or other separate support with the same height).

**d** Trimming on front part of Ultrotome is advised for work with single specimens, especially when reshaping for further sectioning. Picture shows specimen being trimmed while specimen holder is placed in trimming stand.
Routine trimming of a number of specimen blocks is more conveniently performed at a separate table in order not to hamper the sectioning work at the Ultrotome (See **e**).
Different types of specimen tips trimmed for sectioning:

**da** Large straight pyramid. Easy to trim, but not as stable and "specimen-saving" as next type.

**db** Small straight pyramid, suitable for ultrastructure investigations when thinnest possible sections are needed.

**dc** Straight roof-shaped tip. Standard type recommended for serial sectioning. Gives ribbons of sections which are "tough", i.e. sections adhere well to one another when floating on liquid surface.

**dd** Straight rectangular pyramid. This type is also suitable for serial sectioning.

**e** Trimming on the accessory box with specimen in orientation head. Head should always be used when pyramid axis desired is not parallel to axis of specimen holder.

**ea** Pyramid axis may form an angle of up to 47° with specimen holder axis.

**eb** Large oblique pyramid. (See **da.**)

**ec** Small oblique pyramid. (See **db.**)
**Note:** Tips shown in **dc** and **dd** may of course be oriented in a similar way as **eb** and **ec.**

**f Trimming Procedure**
Block is now assumed to have been oriented so that axis of pyramid to be trimmed will be vertical. Recommended hand position (for right-handed operators): Grasp the knife holder lengthwise between the right thumb and forefinger. Rest both wrists on the hand-supporting pad and the small and ring fingers of right hand on the trimming stand (when being used). Hold the specimen holder between the left thumb and forefinger and let the right thumb rest on the left thumb and the right middle finger on the left forefinger. While cutting the right forefinger will now slide along the right middle finger.

**fa** Step by step trimming of large straight pyramid. (See **da.**)

1 Specimen is uncovered at the point where the top of the pyramid is intended to be located.

2&3 Two opposite surfaces of the pyramid are cut (cuts I and II). Microscope pictures (20x) show the result after each cut.

4 Perspective view.

5&6 Last two surfaces are cut (cuts III and IV).

7 Perspective view of large straight pyramid.

**fb** Step by step trimming of small straight roof-shaped tip. (See **dc.**)

1 Specimen is uncovered at the point where the top of the roof is intended to be located.

2&3 Two opposite surfaces of the roof are cut (cuts I and II). Microscope pictures (20x) show the result after each cut.

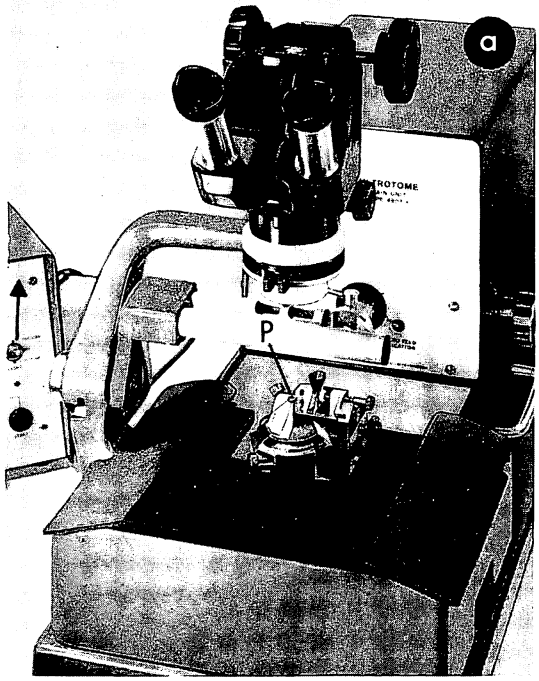
4&5 Cuts III and IV. Do not cut too deep. Stop just before the knife reaches the cuts I and II. Pull away loose parts by lifting upwards and outwards.

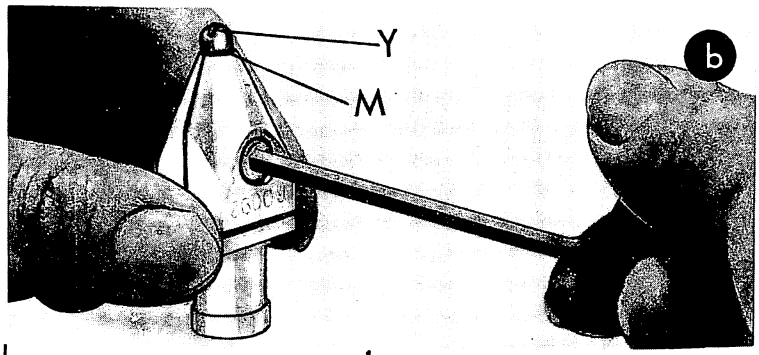
6 Perspective view.

7&8 The last two roof surfaces are cut (cuts V and VI).

9&10 Cuts VII and VIII are made. (See steps 4&5 above).

11 The small rectangular roof-shaped tip is now ready for sectioning.




ba

| Q | Ø m/m | |
|---|---|---|
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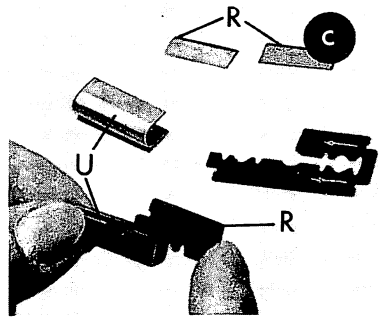
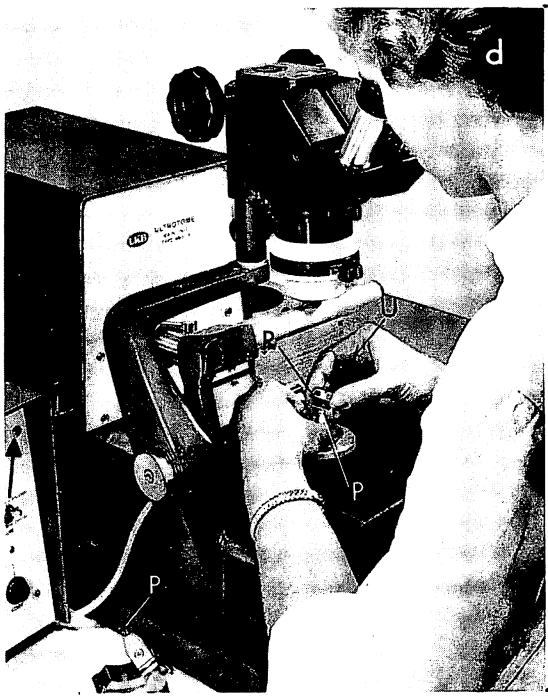
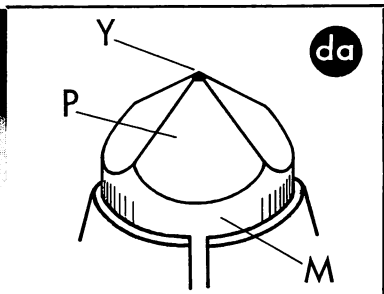
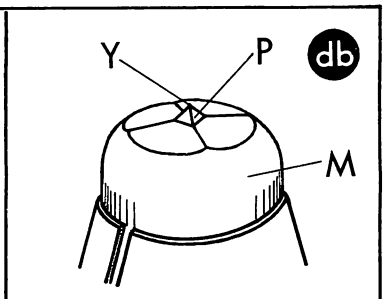
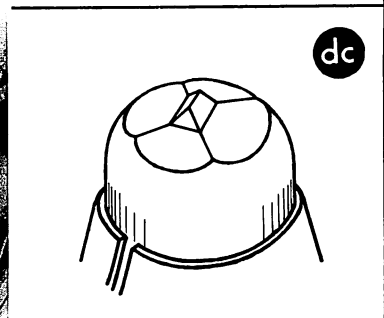
| 0 | 7,2 | F |
| 1 | 6,3 | E |
| 2 | 5,6 | D |
| 4 | 4,8 | B |

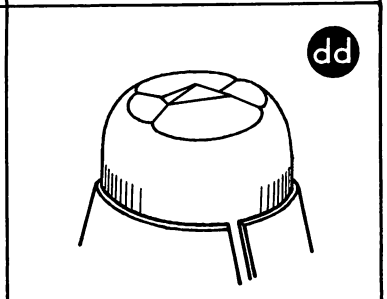


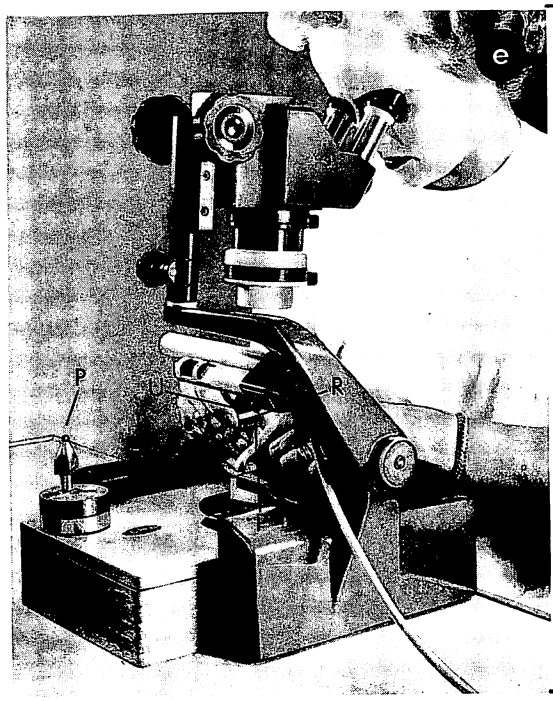



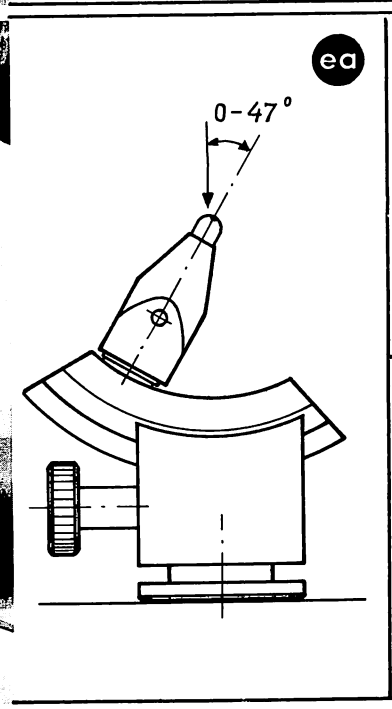



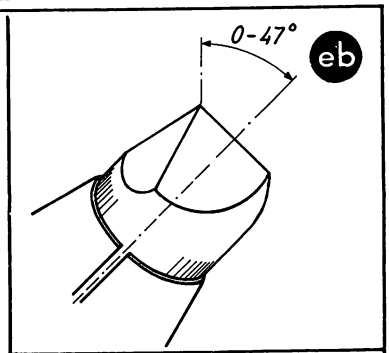



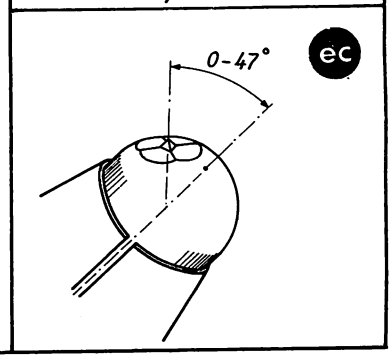



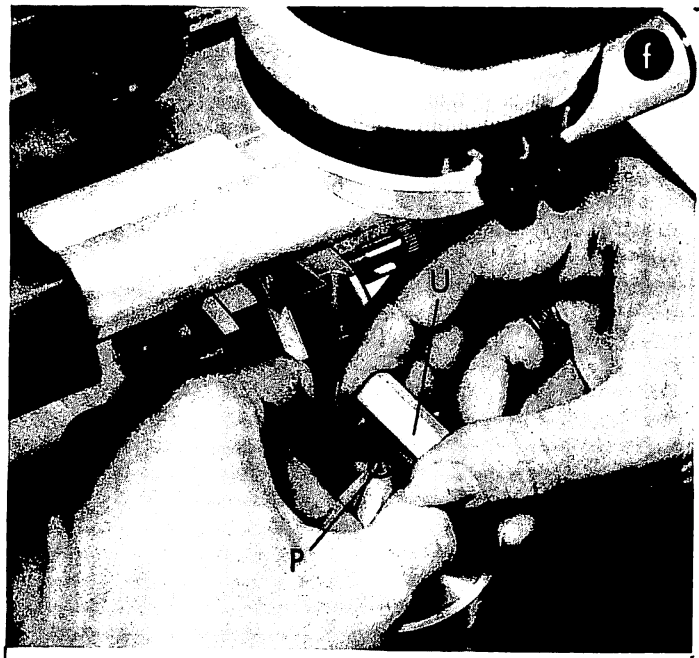

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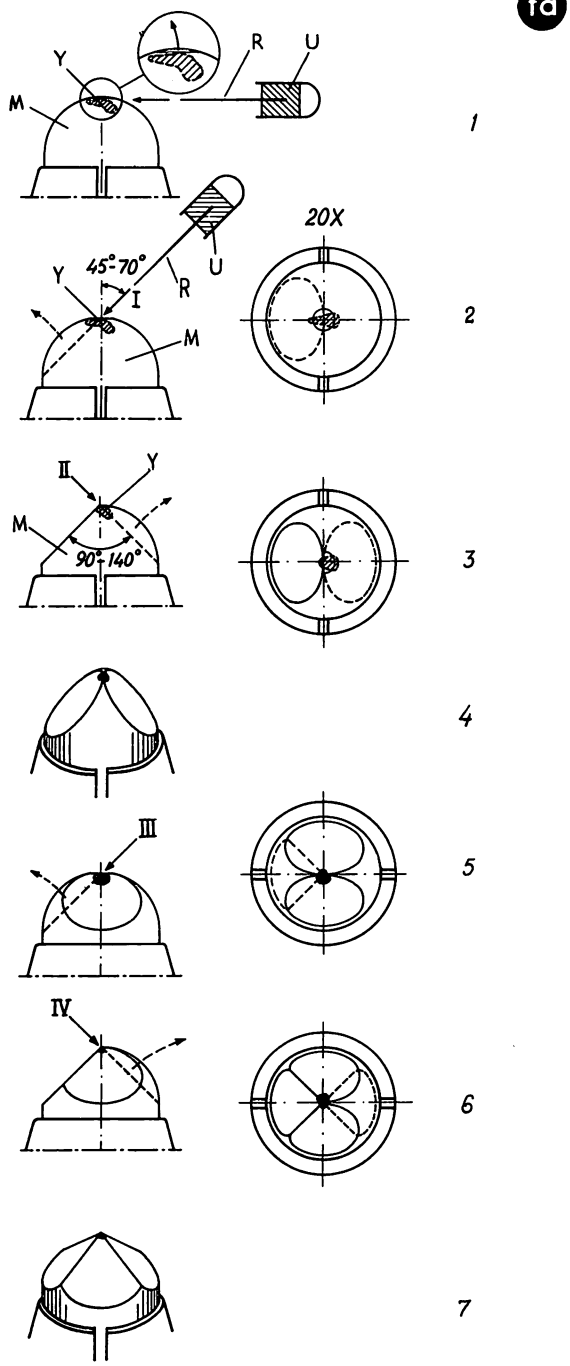
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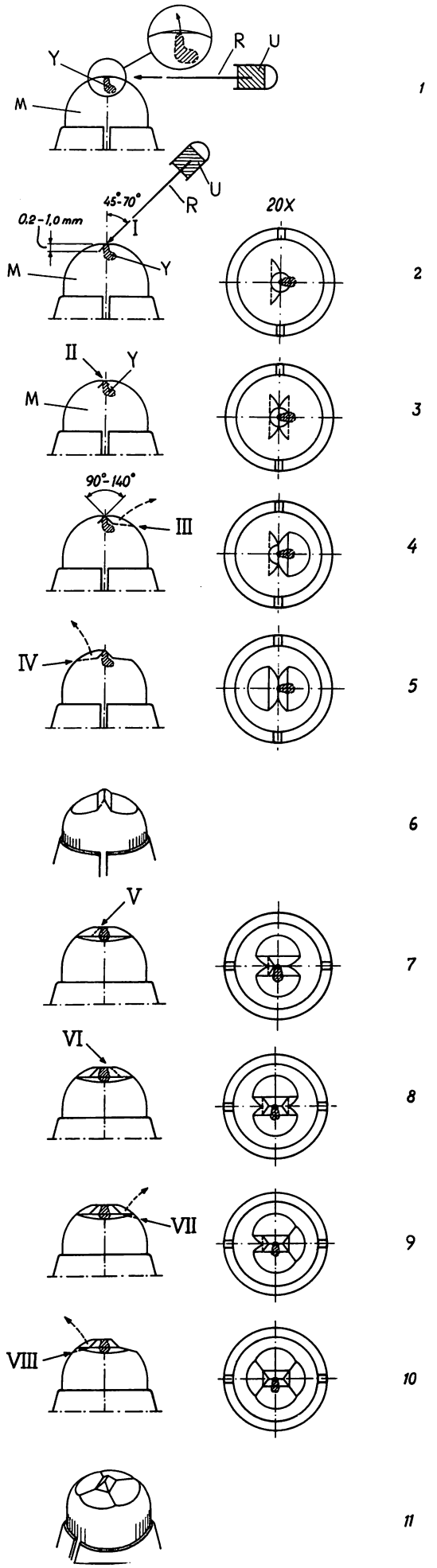
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VII
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VIII
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11

# Glass Knife Production and Inspection

*The following text refers to illustrations marked VII B 2: a–jd*

## a–b Strip Production

Use high quality plate glass, as free from internal stresses as possible.

**a** Score lightly but firmly a number of lines parallel to one edge and 25 mm (1 inch) apart. All lines L1 must be scored on the same side of the plate.

**b** Put plate on a flat rigid support with scored side upwards. Place a match or similar object underneath plate just under and at the end of a score and press down firmly as shown.

**ba** Fracture of a usable strip should have only short and minute flaws emerging from a fine and smooth score.

**bb** A strip showing large flaws should not be used. Score was probably too deep and/or too ragged.

## c–d Knife Production

**c** Put a well cleaned glass strip with scored side downwards on a flat rigid support. Score as shown.

**ca** "Knife score" (L2) should start a small distance (about 1 mm) from corner of strip and be directed so that, if prolonged, it would meet long side of strip just below the corner. (See illustration **da**.)

**d** Remove triangular end piece *essentially* by *traction* and only *slightly* by *bending downwards*. Parallel-jawed pliers should be applied as shown in illustration **d**. In **da** the pliers should be applied nearer to the lower corner.

**da** Scrap the corner-piece just detached. At upperleft corner of strip, knife edge proper has now been formed and should not be touched.

## e Knife Detachment and Preliminary Inspection

**ea** Draw a "cross-score" (L3).

**eb** Pull off knife (be sure not to touch free edge).

**ec** Some typical shapes of knife edges:

1 Knife edge is straight with no or only a very small "horn" at right. Usually the best type.

2 Knife edge is slightly convex. Very often quite good.

3, 4&5 Knife edges which are concave, slanting and/or excessively horned are unsuitable.

**ed** Actual knife angle at edge is usually somewhat larger than angle aimed at while scoring (e.g. 45°).

## f Rough Estimation of Knife Angle

Put knife with "knife score" L2 downwards on an "angle gauge" as shown. The gauge should have a number of fine straight lines forming different angles to a common "zero" line.

**fa** Fit knife edge to gauge so that zero line is tangent to knife surface (at L2) at the very edge. Estimate which line coincides with opposite knife surface (at L1). (In the case shown, knife angle is 49°.)

## g–i Trough Mounting

**g** Put metal trough (T) on knife (K). No part of trough should protrude beyond knife edge (Z) or the "vertical" surface. See dashed line indicating suitable position of trough lips.

**h** Put a small piece of wax (W) in trough so that it makes contact with a wall.

**i** Heat rear rim of trough cautiously until wax melts and by capillary forces is drawn into narrow space between glass and trough. When cool, wax forms a rigid and tight joint between trough and knife.

## j Final Estimation of Knife Quality

The following estimation is also included as part of the routine sectioning work and may thus be postponed.

Mount knife as shown in VII B 3: **e** and **ea**.

Focus microscope lamp on knife edge, the microscope stand having been moved horizontally until arm axis coincides (approx.) with knife edge. Adjust incident angle of light by moving lamp as shown.

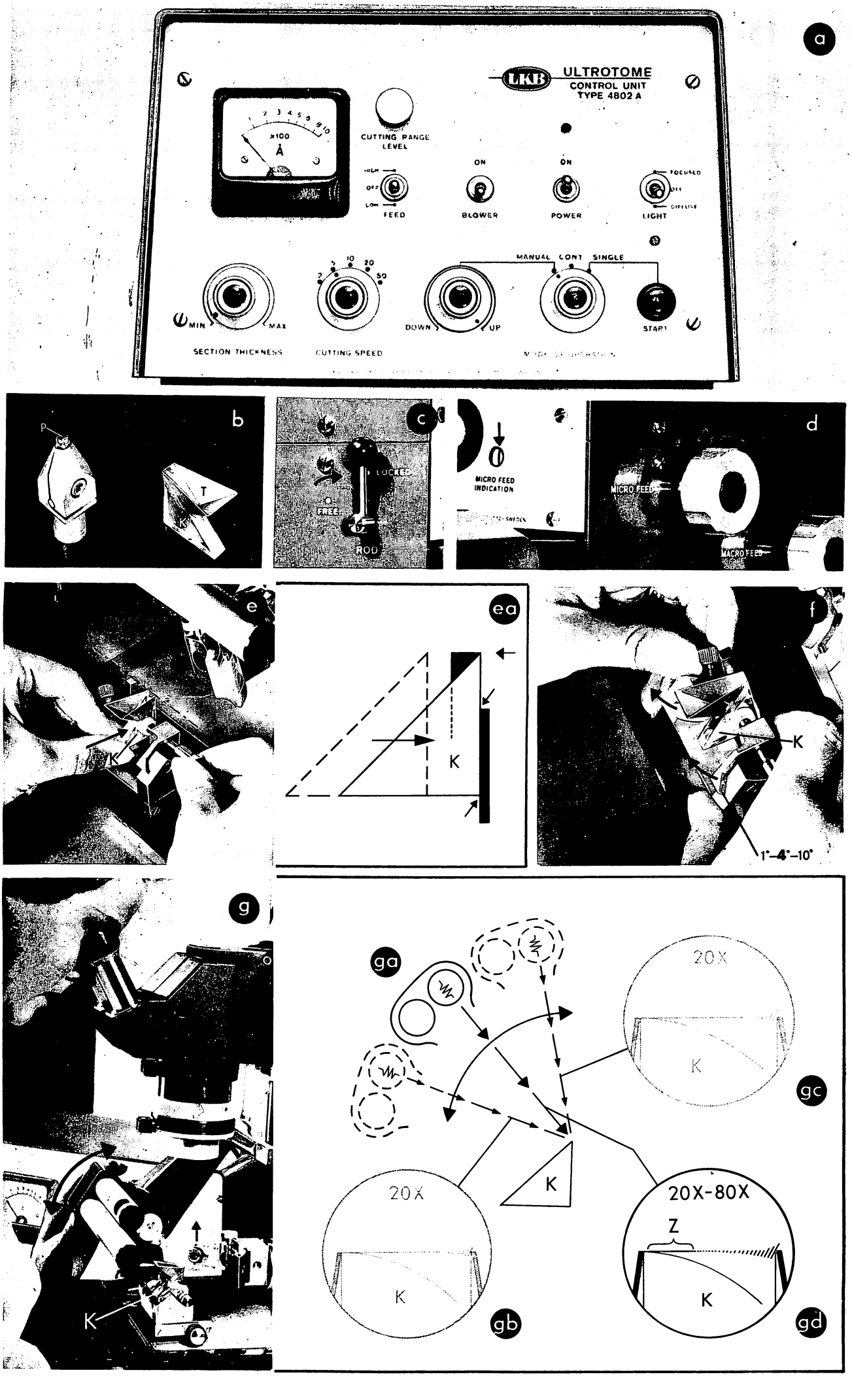
**ja** At a certain middle position, a very good dark field effect will be obtained.

**jb** A too low lamp position will give strong disturbing reflexes from the surface near the edge — the latter then will appear as a long unbroken line of light.

**jc** A too high lamp position will give a similar appearance of the edge as in **jb** but there will be no strong reflexes.

**jd** Lamp position is correct when edge appears as a *very thin unbroken line* and when this line has a *minimum length*. Usable part of knife edge is marked Z in diagram.

**Note:** Thanks to the lamp arrangement which gives the dark field effect a magnification of 80x is quite sufficient for knife edge evaluation.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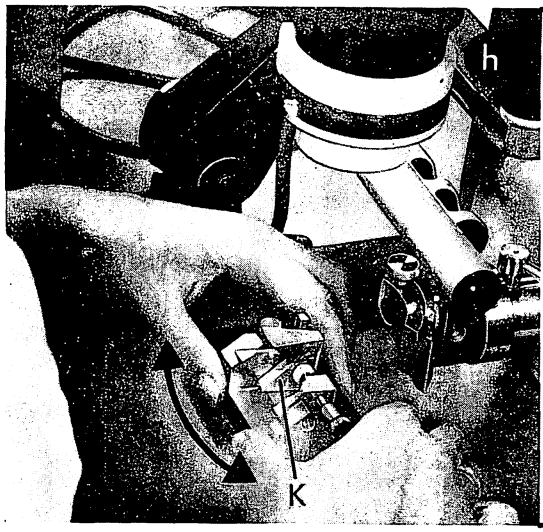
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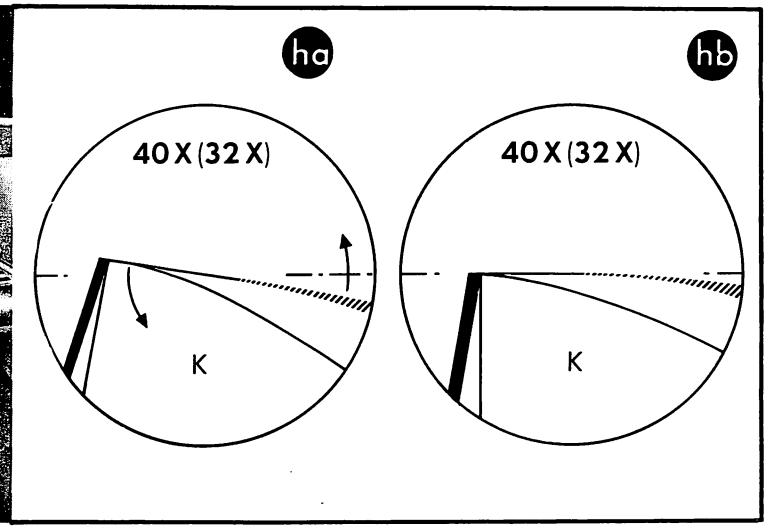
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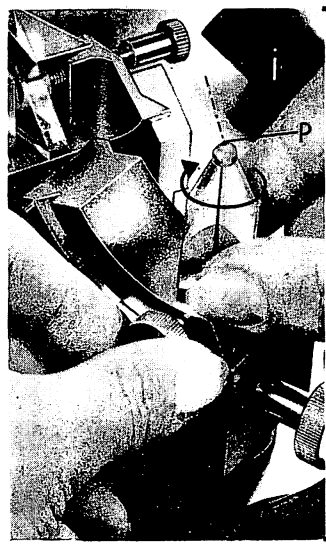
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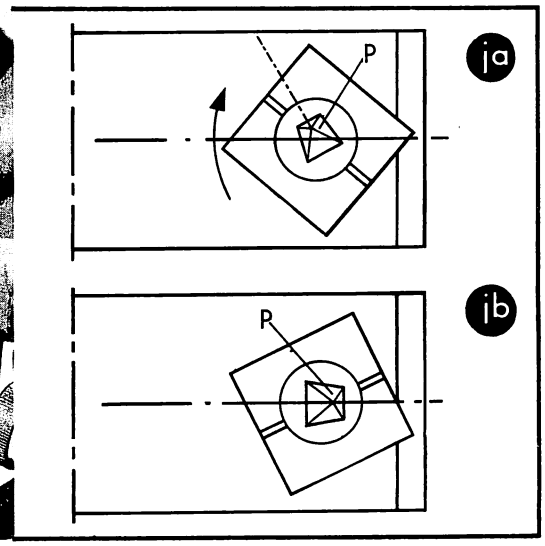
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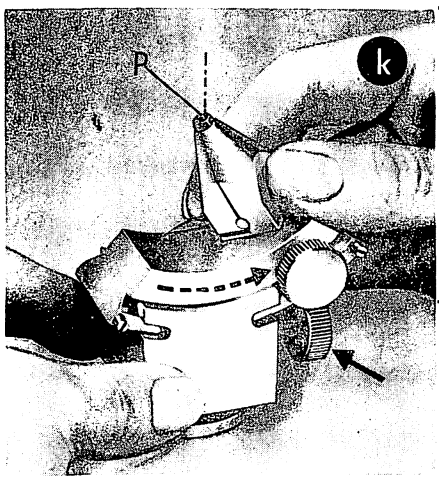
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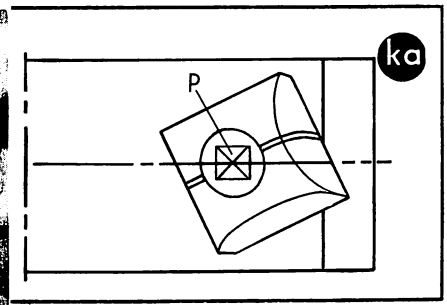
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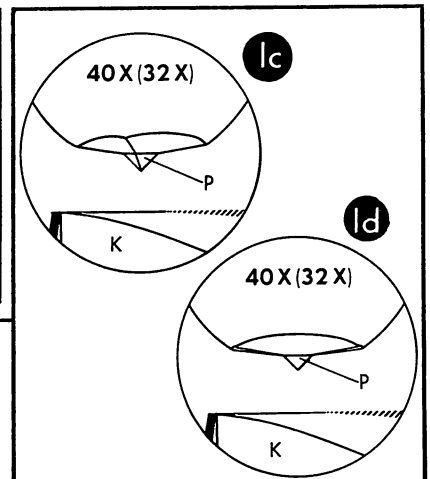
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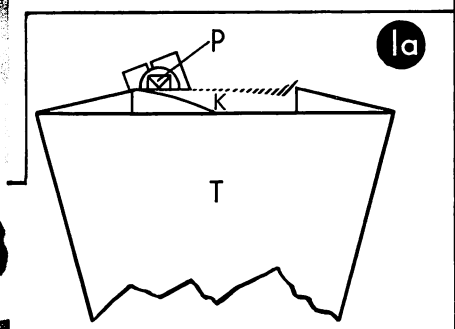
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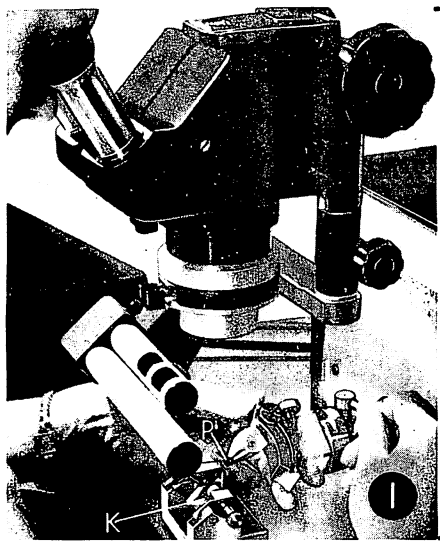
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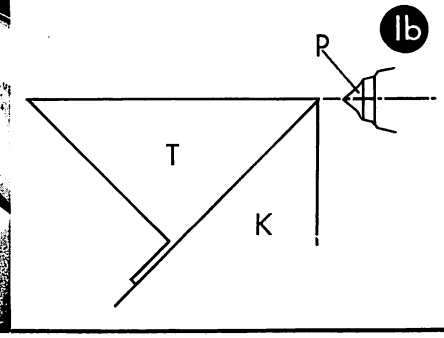
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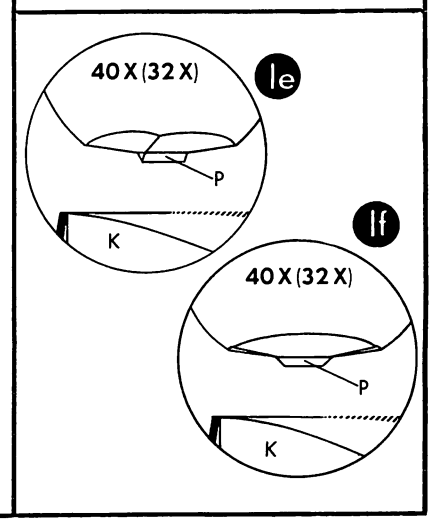
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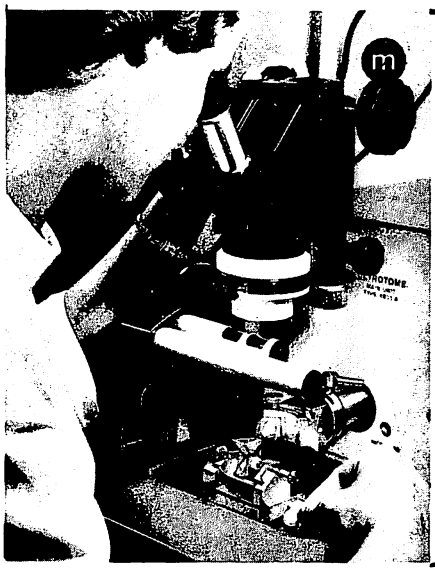
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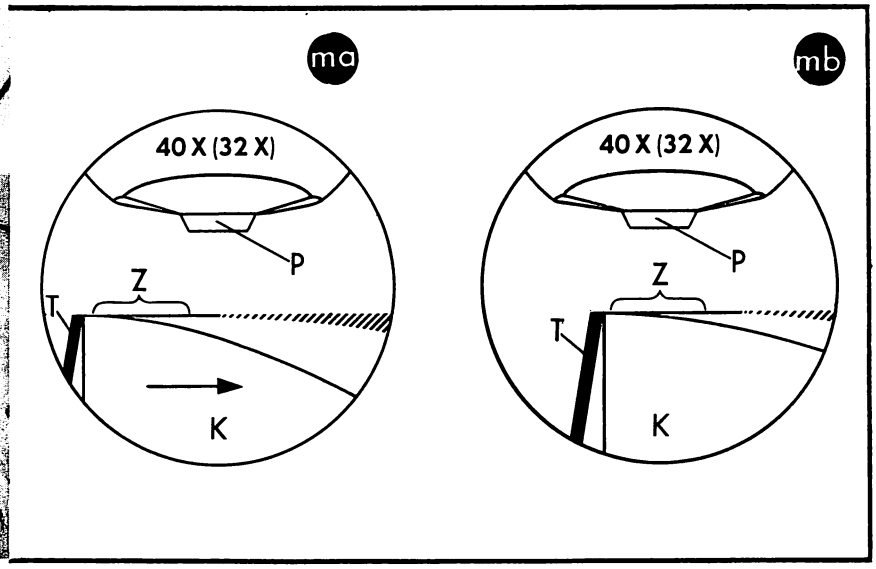
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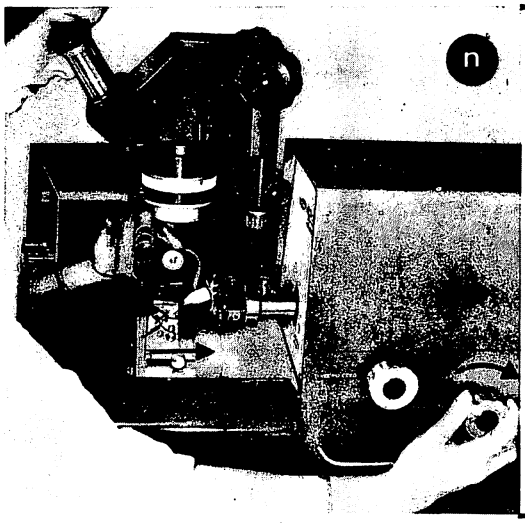
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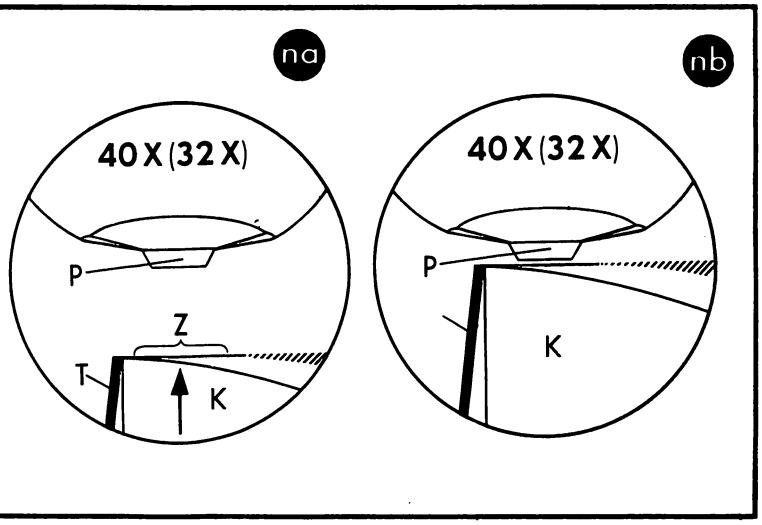
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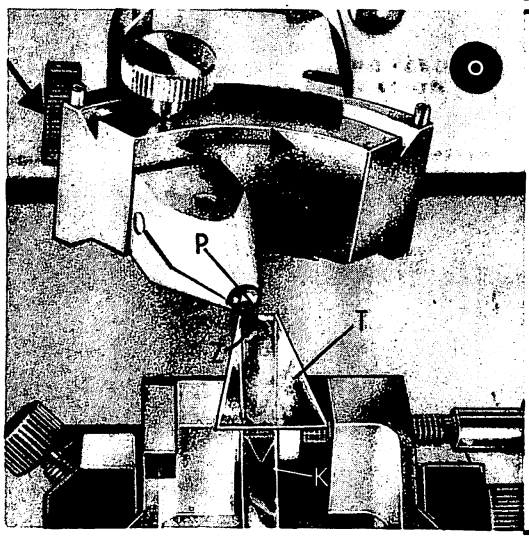
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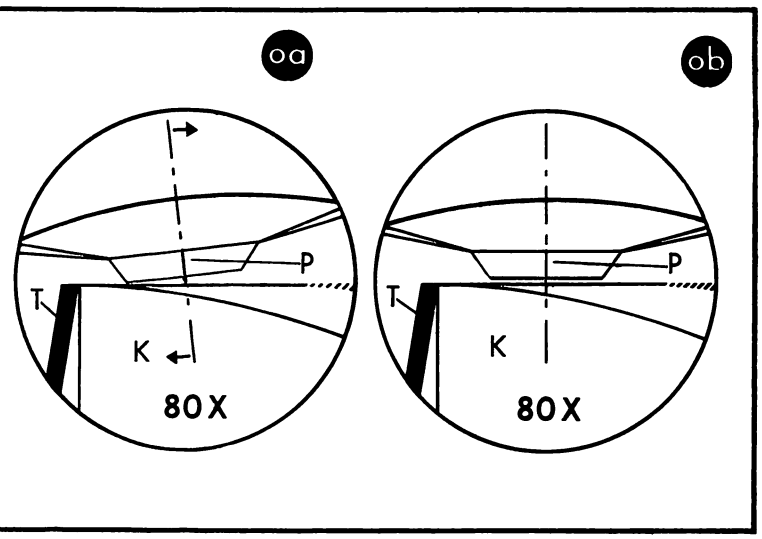
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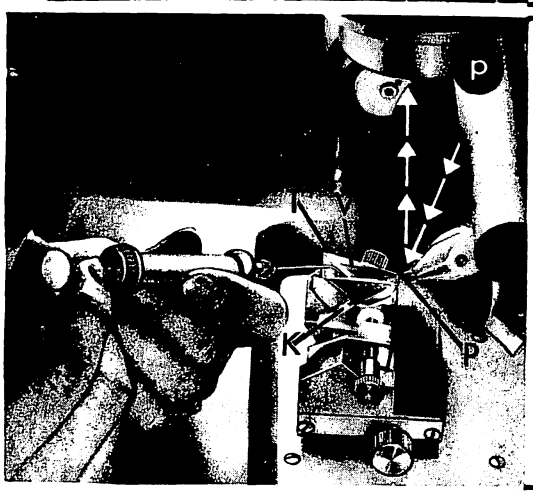
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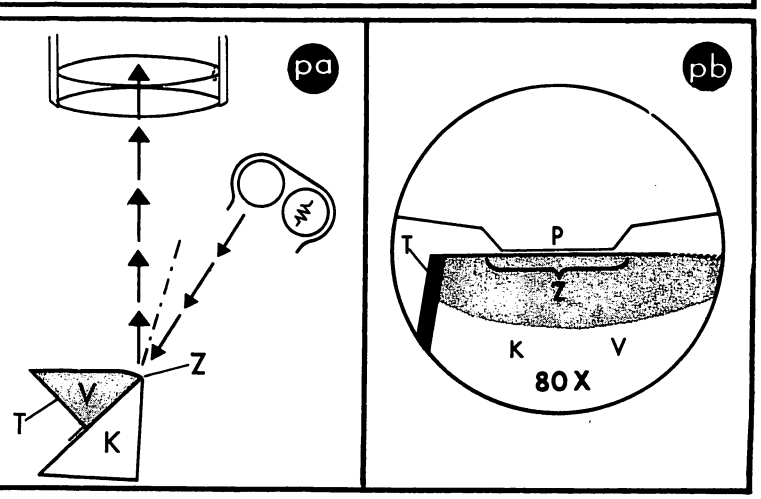
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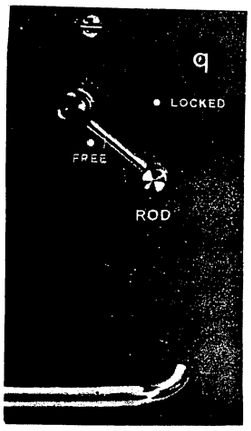
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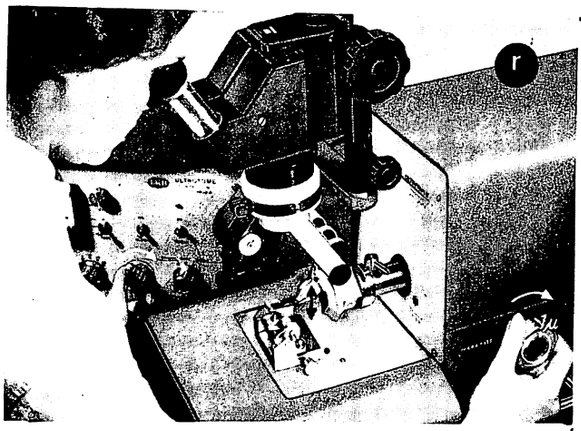
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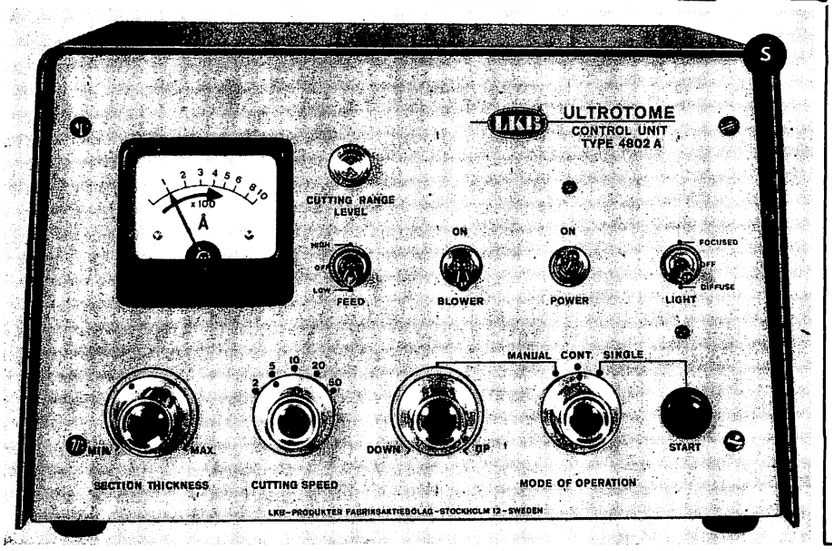
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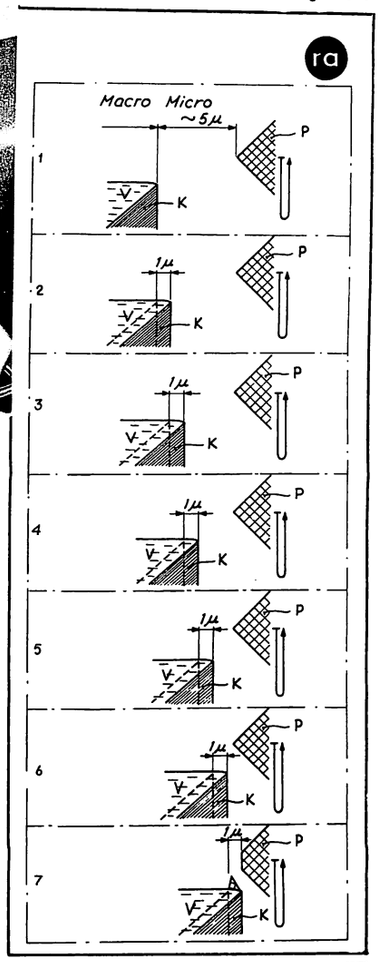
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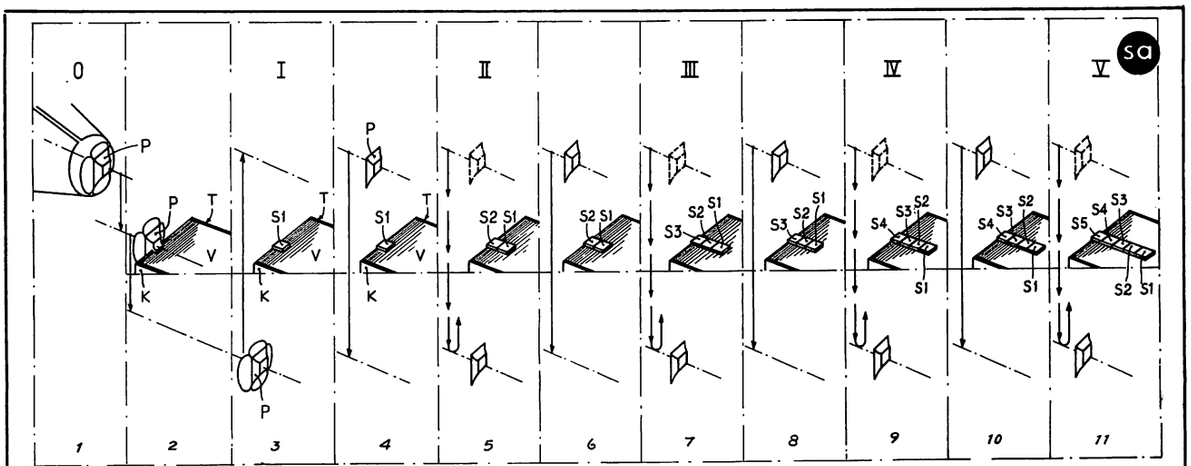
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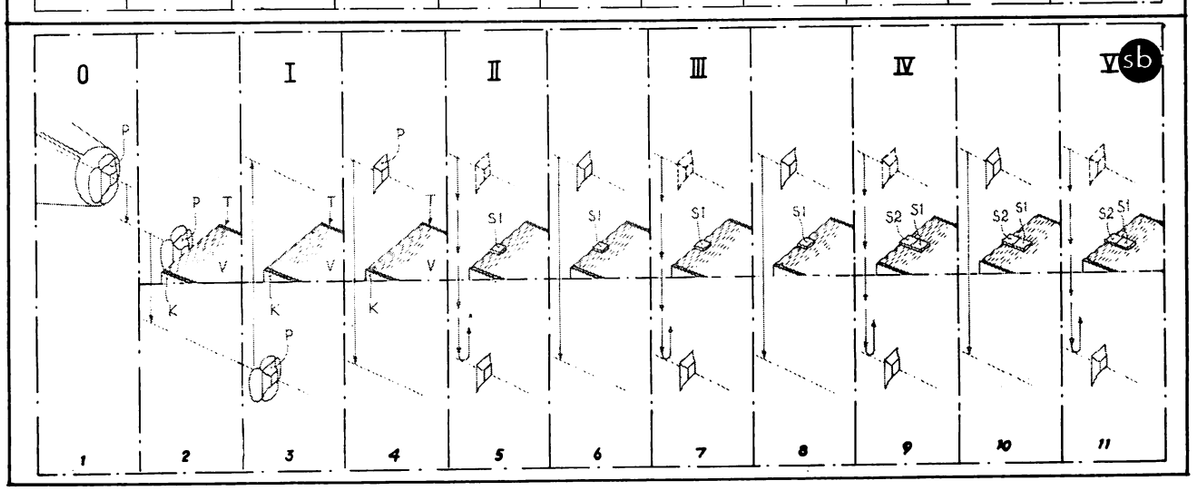
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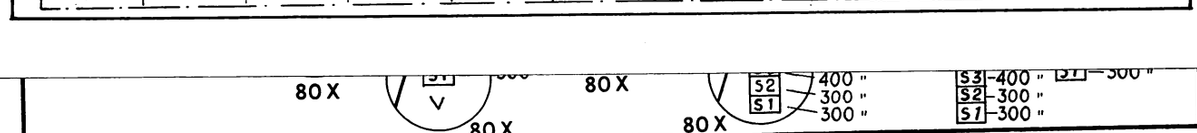
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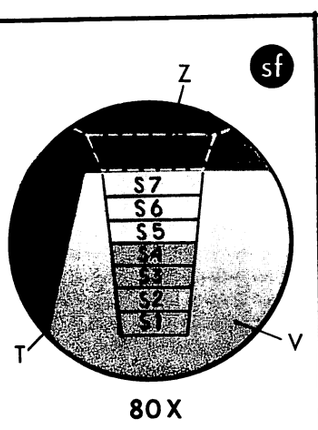
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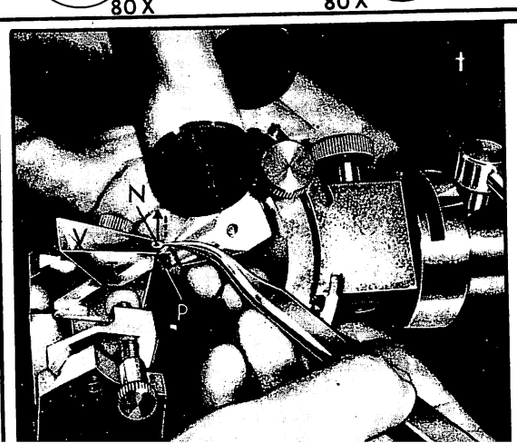
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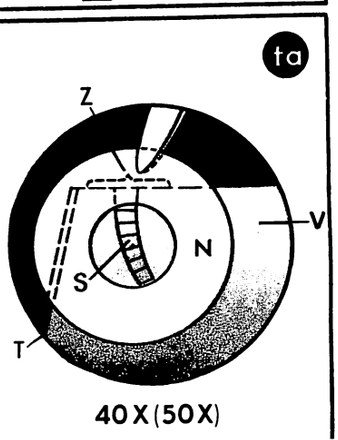
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# Sectioning

*The following text refers to illustrations marked VII B 3: a—ta*

**a—d Initial Conditions**

a Switches and knobs set as shown.

b Trimmed specimen (P) in holder (see VII B 1). Knife (K) with trough (T) (see VII B 2).

c Specimen arm locked.

d "MACRO FEED" and "MICRO FEED" knobs rotated counter clockwise as far as possible.

**e—h Knife Mounting**

e Raise knife height reference rod (to the left of knife position).

ea Push knife against black guide plate. Adjust height of knife edge until it is level with top surface of height reference rod. Clamp knife in correct position.

f Adjust clearance angle of knife, usually to about 4°.

g Inspect knife edge — see VII B 2: j—jd.

h Turn knife support until knife edge is perpendicular to the axis of the specimen arm. Use magnification 40x.

ha Knife edge incorrectly aligned (microscope view).

hb After rotation of knife support, edge looks "horizontal" in field of view.

**i—k Specimen Mounting**

i Plug the specimen holder into the hole in arc of specimen orientation head with latter resting on its circular base.

j Turn specimen holder until pyramid (P) is suitably located. Lock.

ja Incorrect orientation: axis of pyramid is pointing away from midplane of arc. Rotate specimen holder so that inclined axis of pyramid points towards nearest end of arc.

jb After rotation of specimen holder, axis of pyramid is parallel to midplane of arc.

k Loosen arc and let it slide in head until axis of pyramid is vertical. Lock.

ka Symmetrical appearance of pyramid when properly oriented (and trimmed).

**l Mounting of Head**

la Push the base of head down in slot on specimen arm and turn it around until the lower face of the pyramid is parallel to knife edge. Arc is horizontal in the case illustrated here.

lb Move head up or down until tip of pyramid is level with knife edge.

lc Microscope view of pyramid before alignment.

ld Same pyramid properly aligned with upper and lower faces parallel with knife edge (see la).

le Microscope view of roof-shaped specimen tip not yet aligned.

lf Same specimen properly aligned: roof edge is parallel to and level with knife edge.

**m—o Knife and Specimen Adjustments**

m Move knife support sideways until usable part of knife edge is properly oriented relative to specimen.

ma Specimen is not in front of usable part (Z) of edge.

mb Part Z is properly oriented in front of tip.

n Turn "MACRO FEED" knob slowly clockwise until knife is very close to specimen tip.

na Too large distance between knife and specimen.

nb Correct distance: a few microns.

o Move arc for final adjustment of roof edge orientation.

oa When a roof-shaped specimen tip is used it is easy to see when the edges are not parallel.

ob Correct alignment.

**p Filling of Trough**

Use e.g. 10—20 % ethanol. Add liquid until level reaches knife edge.

pa Move lamp backwards. Set microscope vertically.

pb Stop filling when bright reflection from lamps spreads out over most of liquid surface in view.

**q Release Specimen Arm** (position "FREE")

**r Approach to First Contact**

Move knife forwards by turning "MICRO FEED" knob (with specimen arm in "UP" position) clockwise and stepwise, e.g. one turn at a time. Move specimen arm down and up by using "DOWN-UP" knob.

ra Set microscope to highest magnification. Watch knife edge approaching specimen tip. Stop knife movement when the first contact has been established. Move rod back to the "UP" position.

**s Sectioning**

**Choose cutting speed:**
Settings 2 and 5 (mm/sec.) for large sections and/or hard materials.
Settings 10, 20 and 50 for medium and small sections and/or soft materials.

**Choose feed:**
Switch on feed current ("HIGH" or "LOW"). Adjust feed by "SECTION THICKNESS" knob.

**Choose mode of operation:**
*Thinnest possible sections:*
"MODE OF OPERATION" switch set to "CONT". Start with minimum feed and increase until a section is produced with *each* stroke.

*Thicker sections:*
(may be obtained in three different ways):

1 Continuous sectioning with "SECTION

THICKNESS" knob set to desired value.

2 Set "MODE OF OPERATION" switch on "SINGLE". Push "START" button at regular intervals, e.g. at a multiple, *n*, of 3 seconds ( $n$ = number of blinks). This will give a section thickness *n* times reading on meter.

3 Turn "MICRO FEED" knob clockwise, e.g. one turn (approx. 1 micron). Use "SINGLE" position and "START" button (thermal feed may or may not be disconnected). "CONT." position may also be used if micro feed movements are performed when specimen arm in its rest position.

**sa** Normal function (properties of knife and specimen meet requirements set by feed chosen). *Each* stroke produces a section.

**sb** Appearance when feed chosen is too low to suit specimen and edge quality. Every *second* stroke produces a section. Remedy: *increase* feed until *every* stroke gives a section. The sections will then be thinner.

**sc** Appearance at start of sectioning in case where knife and specimen are such that it is impossible to get thinner sections than 250 Å. 100-Å feed gives sections of about 300 Å every third stroke. Remedy: see **sd**.

**sd & se** As an example, increasing feed to 200 Å will give sections about 400 Å thick every second stroke. A further increase of feed to 250 Å will give 250-Å sections at each stroke. 250 Å feed setting will continue to give sections every stroke until knife has lost its sharpness due to wear or contamination. Then feed may be increased until sections are produced at every stroke.

**sf** Inspect sections at 80x magnification in reflected light while they are floating on liquid surface. They should appear as some shade of gray. The thinner the section, the more its surface will resemble the liquid surface. When thickness is 700–800 Å, they may have a yellowish tint.

## t Collecting Sections

Catch sections on grid-, slit- or hole-type aperture plate (N) suitable for electron microscope used. Lower the plate (covered with e.g. Formvar) carefully down over sections on liquid surface just to make contact and then lift cautiously up again. Sections now adhere to film on lower side of plate ("grid").

**ta** Illustration shows hole-type aperture plate in use for catching a series of sections. This type of aperture plate is especially suitable for serial sectioning work. Carbon coating is recommended whenever large film areas are used.

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